ejpmr, 2023,10(1), 360-363

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

PIEZOELECTRIC MATERIALS FOR SONODYNAMIC THERAPY IN TUMOUR TREATMENT

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Article Received on 20/11/2022 **Article Revised on 10/12/2022** **Article Accepted on 30/12/2022**

ABSTRACT

The conventional therapy of cancer has been replaced with non-invasive approaches, among which sonodynamic therapy (SDT) emerged as a golden technique that effectively destroys cancerous cells. It expedites the production of reactive oxygen species (ROS) intracellularly allowing the high specificity in tumour cell destruction. In this study, we use BCTZO piezogel as piezoelectric materials in SDT on MCF-7 cells. **Methodology:** The MCF-7 cells were treated using three different concentrations (8, 16 and 32 µg/mL) of BCTZO and BCZTO-Ag for 48 h. Hoechst 33342, which is a specific strain used for AT-rich regions of double-stranded DNA was utilized. The cells were incubated for 15 min with Hoechst 33342 dye (5 µg/mL in PBS) and subsequently visualized using a BHZ, RFCA microscope equipped with a fluorescent light source with an excitation wavelength of 330 nm and a barrier filter of 420 nm. **Conclusion:** Outcomes of Hoechst staining carious out on MCF-7 cells indicated that the BCZTO-Ag US has the highest apoptotic effect when compared with the cell treated with BCZTO, US and control line respectively. Therefore, BCZTO-Ag US could be the best therapeutic approach for cancer chemotherapy.

KEYWORDS: Sonodynamic therapy, Piezoelectric materials, MCF-7 cells, BCZTO-Ag US.

INTRODUCTION

Cancer is one of the most dangerous diseases globally and the second leading cause of death in the world. The major contemporary conventional therapeutic approach includes radiation therapy, surgical treatment and chemotherapy. However; it remains the key choice of treatment for most patients.[1] These cancer therapies for the medical mission are still the most challenging and clinical methods await minimally invasive therapeutic modalities. Development of non-invasive novel treatments to avoid the severe unwanted pain of patients and prevent the patient from harmful side effects of radiation therapy, surgical and chemotherapy.[2] The systemic toxicity, low selectivity, drug resistance and potential long-term side effects are prominent drawbacks of the conventional treatments. These unwanted side effects limit the treatment prospects of traditional therapy for tumours. Hence, there is an urgent need for minimally invasive and highly selective antitumor treatment for the patient.[3]

SDT is the alternative method to treat cancer patients. In some dynamic therapy, the tumour is exposed to ultrasound radiations to induce the production of reactive oxygen species (ROS) intracellularly which subsequently results in cellular damage. The ultrasound employed in SDT can penetrate deep into biological tissue while non-invasive has a low tissue attenuation coefficient. Although the efficacy of the SDT in tumour treatment is low, hence any improvement in the efficacy is more than welcome.[4,5]

Recently, the inclusion of sonosensitizers in the SDT has evolved as a novel method to increase the effectiveness of SDT in tumour treatment. Ultrasound waves activate the sonosensitizers and focus on sites of tumour.[6]Some researchers proved that the inclusion of piezoelectric materials in SDT increases its effectivity, as piezoelectric materials possess the ability to generate surface potential when exposed to mechanical stress. This surface potential can trigger the micro-electrolysis of water molecules and generate ROS as an intermediate in an aqueous system.[7]

Sonosensitizers activates by ultrasound radiation, leading to the regression or complete eradication of cancerous tissue when administered systematically or locally into the tumour tissue.[8]

The use of BCTZO piezogel in SDT has not yet been reported. Therefore; our research focus on improving the therapeutic potential of SDT under catalytic mediation by piezoelectric $Ba_{0.85}Ca_{0.15}Ti_{0.9}Zr_{0.1}O_3$ (BCTZO) nanogel. The piezoelectric effect of Piezogel enhances

the production of ROS inside the tumour cell (*in situ*). BCTZO has the properties like high d_{33}, and high Curie temperature. The performance of gel would be further enhanced by incorporating silver (Ag) in BCZTO which may increase the time of electron-hole separation during performance upon ultrasound exposure.

METHODOLOGY

Synthesis of BCTZO

A standard solid-state reaction technique was used to produce Ba0. 85Ca0. 15Ti0. 9Zr0. 1O3. High-purified oxides of barium carbonate (BaCO3 (Fisher)), calcium carbonate (CaCO3 (Fisher)), zirconium dioxide (ZrO2 (Fisher)), and titanium dioxide (TiO2 (Fisher)) were combined in a mortar-pestle in a 1:1 mixture. After that, the powder was thermally treated in the furnace for 4 hours at 1300 C. The temperature of calcination had already been mentioned in previous papers. Powder X-ray diffraction analysis was used to look for phase formation as in calcined powder that was hand-ground using a mortar-pestle.

When BCZTO powder is obtained after calcination, the process for silver (Ag) mediated BCZTO gel starts. In 30 ml of ethylene glycol solution, calcined BCZTO powder (0.5 g) and AgNO3 (Himedia) (0.5 g) were dissolved. A magnetic stirrer was used to mix the suspension for 2 hours. Following that, the raw material was filtered via filter paper. The crude powder was cleaned with water and acetone before being dried in an oven at 100 C for 12 hours to achieve the desired Ag-loaded BCZTO particles. Further, the synthesis of BCZTO gel and Silver loaded particles is evaluated using different novel characterization techniques.

Synthesis of BCTZO piezogel

Momentarily, 100 mg of chitosan (deacetylation degree: 75 – 85 and thickness: 0.2 – 0.8 Pa·s at 25 °C, Sigma-Aldrich) was disintegrated with a steady blending at room temperatures in 4 mL of acidic corrosive (0.1 m), until the arrangement was clear. Consequently, 600 mg β-GP (β-Glycerophosphate, Sigma-Aldrich)) was disintegrated into 1 ml deionized water with a 0.22 µm needle channel. Until combined as one, the two arrangements were cooled to 4°C for 20 minutes. To get a homogeneous fluid arrangement, β-GP has then been tenderly added to the chitosan arrangement in an ice shower under moderate tumult drop side. The last arrangement was ready with 2% chitosan and 12% (w/v) β-GP. By embedding T-BTO nanoparticles into the chitosan hydrogel with a volume proportion of 1:9 as a clear, an injectable T-BTO-Gel was ready. The mixture was blended homogeneously with a vortex for two minutes. Till use, the last arrangement was held at 4°C.

Evaluation of Antitumor therapy by $Ba_{0.85}Ca_{0.15}Ti_{0.9}Zr_{0.1}O_3$ (BCTZO) mediated Piezogel

MTT assay

Briefly, 24 h before treatment, MCF-7 cells (5 × 104 cells/mL) were seeded in a 96-well plate. Dissolved compounds in RPMI were used in various concentrations (from 7.8 to 500 µg/mL). After 72 hrs each well of plates was added 20 µL of MTT solution and then the plates were incubated for a further 4 h. In the next step, 150 µL of DMSO was put into each well and incubated for 10 min to solve the purple formazan crystals. The dose-response curves were mapped to obtain IC50 values and identify the best active fractions or pure compounds.

Hoechst 33342 Staining

Using a functional vital dye the classical morphological criteria, the quantification and determination of cell death notation were carried out. The MCF-7 cells were treated using three different concentrations (8, 16 and 32 µg/mL) of BCTZO and BCZTO-Ag for 48 h. Hoechst 33342, which is a specific strain used for AT-rich regions of double-stranded DNA was utilized. The cells were incubated for 15 min with Hoechst 33342 dye (5 µg/mL in PBS)) and subsequently visualized using a BHZ, RFCA microscope (Olympus, Tokyo, Japan) equipped with a fluorescent light source with an excitation wavelength of 330 nm and a barrier filter of 420 nm.

RESULT AND DISCUSSION

MTT assay

Breast Cancer cell lines (MCF) are selected for the MTT studies. MTT solution was prepared in different concentrations from 50 micrograms per ml to 200 micrograms per ml. Cell lines that received BCZTO and BCZTO-Ag were reported to have only 5-10 % of cell viability. While the cells that received BCZTO-US were reported to have around 60% of cell viability. When BCTZO-Ag were administered to the cells having ultrasounds; it has shown a higher degree of cell apoptosis. Cell viability has been reported up to 17 %in this category. In BCZTO-Ag treated cells, a higher number of apoptotic cells have been reported because of the electron-holding capacity of Ag. The piezocatalytic therapy by T-BTO-Gel combined with US irradiation provides a highly promising potential for tumour eradication in vivo.

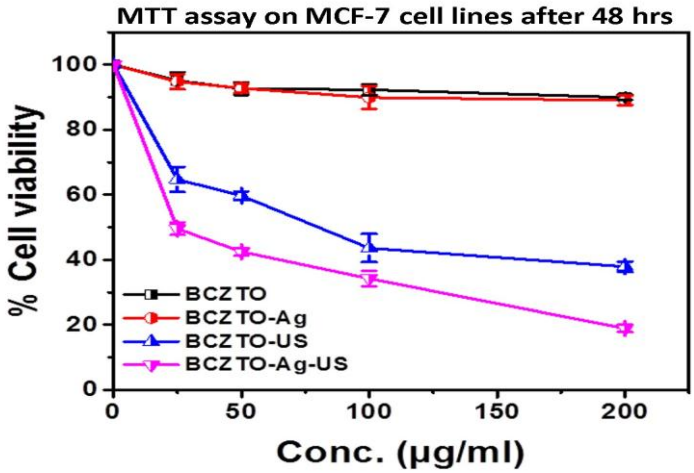

Figure 4: Demonstration of % cell viability and apoptosis caused by BCZTO and BCZTO- Silver loaded cells in normal conditions and US irradiated conditions respectively.

Hoechst 33342 Staining

Using a functional vital dye the classical morphological criteria, the quantification and determination of cell death notation were carried out. The MCF-7 cells were treated using three different concentrations (8, 16 and 32 µg/mL) of F13b1/PV-EA for 48 h. Hoechst 33342, which is a specific strain used for AT-rich regions of double-stranded DNA was utilized. The cells were incubated for 15 min with Hoechst 33342 dye (5 µg/mL in PBS)) and subsequently visualized using a BHZ, RFCA microscope (Olympus, Tokyo, Japan) equipped with a fluorescent light source with an excitation wavelength of 330 nm and a barrier filter of 420 nm.

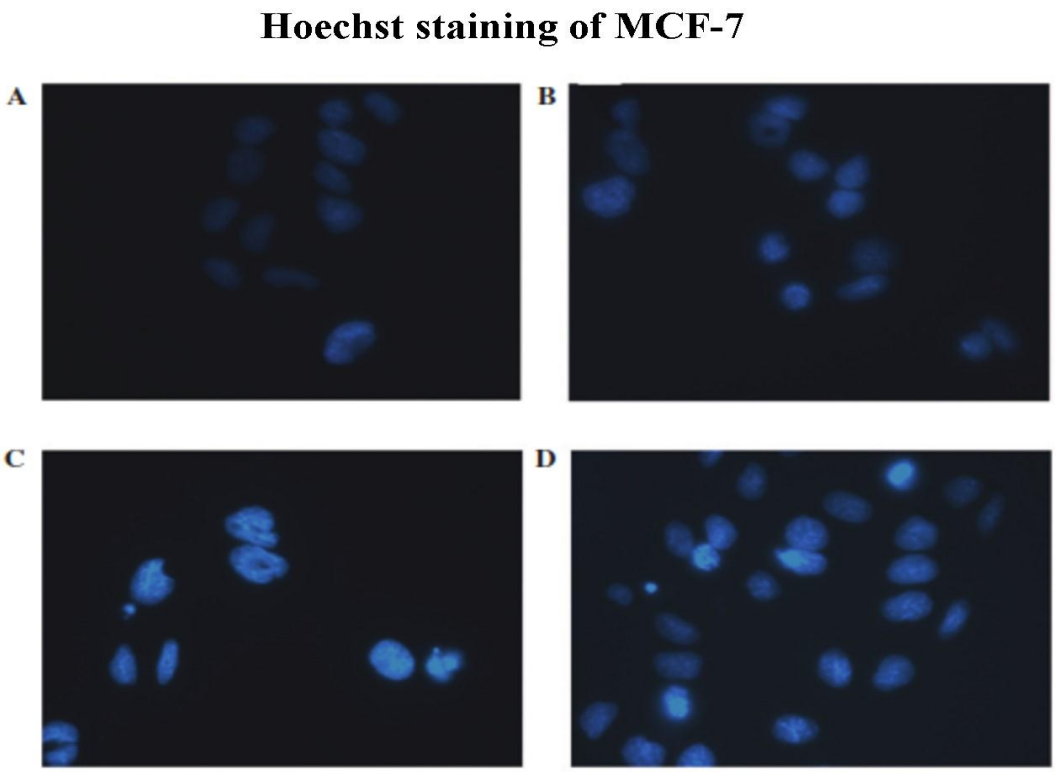

Figure 5: Outcomes of Hoechst staining carious out on MCF-7 breast cancer cells. The figure is demonstrating the number or quantity of apoptotic cells after the MTT assay. A) Control group indicates a lesser number of apoptotic cells. B) Group of cells that only received US irradiations. C) BCZTO received cells which were irradiated using the US. D) US irradiated cells received silver-loaded BCZTO.

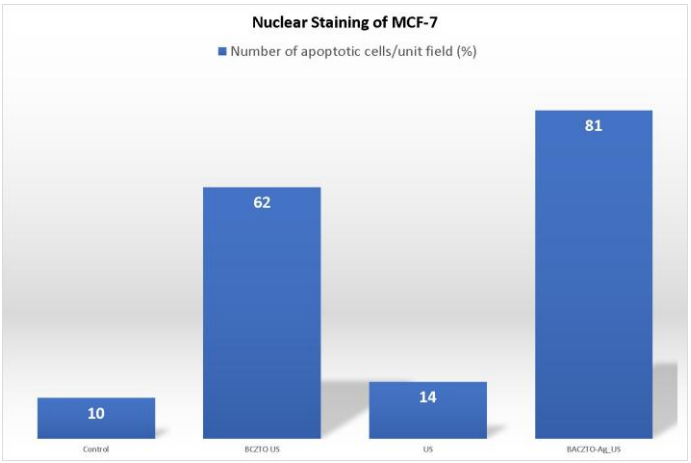

Figure 6: Presented quantitative graph is the outcome of the MTT assay after Hoechst staining. The number of apoptotic cells has been shown in the given graph for control, US, BCZTO-US and Ag-treated BCZTO-US cells.

CONCLUSION

Outcomes of Hoechst staining carious out on MCF-7 cells indicated that the BCZTO-Ag US has the highest apoptotic effect when compared with the cell treated with BCZTO, US and control line respectively. Therefore, BCZTO-Ag US could be the best therapeutic approach for cancer chemotherapy.

ACKNOWLEDGEMENT

We acknowledge the contributions of the entire academic staff of the School of Pharmacy Sharda University India.

Conflict of interest

Nil

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